

Morphine-Induced Straub Tail Reaction and Spinal Catecholamine Metabolite Content: Antagonism of Naloxone to Morphine-Induced Effects in Mice¹⁾

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(Received November 7, 1977)

The effects of morphine on the 3-methoxytyramine (3-MT) and normetanephrine (NM) level of various brain sites and two parts of spinal cord were investigated in mice with reference to our prior data on the effects of catecholaminergic agents on the morphine-induced Straub tail reaction (STR). Morphine (10 mg/kg) increased the 3-MT and NM content in the thoracic and lumbar regions of the spinal cord significantly, without affecting the 3-MT or NM content of the various brain sites. Naloxone (1 mg/kg) antagonized the morphine-induced increase in the 3-MT and NM in the thoracic and lumbar cord. These results suggest that morphine accelerates the release of spinal dopamine and norepinephrine. The morphine-induced STR might be due to the acceleration of catecholaminergic neuronal activities in the spinal cord in mice.

Keywords—morphine; Straub tail reaction; naloxone; 3-methoxytyramine; normetanephrine; catecholamine metabolites; spinal cord; mice

Previously, we have shown inhibitory effects of central muscle relaxants and various amino acids on the morphine-induced Straub tail reaction (STR).³⁾ Furthermore, it is suggested that the development of the STR is related to biogenic amines in the central nervous system (CNS), although morphine does not alter the content of dopamine (DA) and norepinephrine (NE) in the brain and spinal cord.⁴⁾ On the other hand, it is known that both DA and NE are converted to 3-methoxytyramine (3-MT) and normetanephrine (NM), respectively,⁵⁾ by the enzyme, catechol-O-methyltransferase which is mainly located extraneuronally in the tissues of animals⁶⁾ in contrast with monoamine oxidase which is distributed intraneuronally.⁷⁾ Thus, the accumulation of 3-MT and NM might reflect the amount of DA and NE released from these catecholaminergic neurons. The present study was performed to determine the effects of morphine on 3-MT and NM content with reference to our prior data on the effects of catecholaminergic agents on the STR. In addition, since naloxone has been reported to antagonize certain stimulant effects of morphine,⁸⁾ the ability of this drug to modify morphine-induced catecholamine (CA) metabolite content was assessed.

- 1) A part of this research was presented at the 97th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1977.
- 2) Location: *Tenpaku-ku, Nagoya 468, Japan.*
- 3) T. Kameyama, T. Nabeshima, and M. Ukai, *Chem. Pharm. Bull.* (Tokyo), **25**, 1837 (1977); T. Kameyama, M. Ukai, and T. Nabeshima, *ibid.*, **26**, 770 (1978).
- 4) T. Kameyama, M. Ukai, and T. Nabeshima, *Jpn. J. Pharmacol.*, **28**, 249 (1978).
- 5) J. Häggendal, *Acta Physiol. Scand.*, **59**, 261 (1963).
- 6) J. Axelrod, H. Weil-Malherbe, and R. Tomchick, *J. Pharmacol. Exp. Ther.*, **127**, 251 (1959); J. Axelrod, *Pharmacol. Rev.*, **18**, 95 (1966).
- 7) C. Schnaitman, V.G. Erwin, and J.W. Greenawalt, *J. Cell Biol.*, **32**, 719 (1967); J.D. Barchas, R.D. Ciaranello, J.M. Stolk, H. Keith, H. Brodie, and D.A. Hamburg, "Biogenic Amines and Behavior," ed. by S. Levine, Academic Press, New York and London, 1972, pp. 235-329.
- 8) F.S. Messiha and J.C. Krantz, Jr., *Arch. Int. Pharmacodyn. Ther.*, **206**, 90 (1973); J. Cott and J. Engel, *J. Neural Transmission*, **40**, 253 (1977).

Materials and Methods

Albino male mice of ddY strain, weighing between 18 and 25 g, were used. Groups of fifteen mice were employed in each experiment. The drugs used were morphine hydrochloride (Shionogi and Co., Ltd.) and naloxone hydrochloride (Sankyo Co., Ltd.). The STR was confirmed according to the modified numerical rating of Juul⁹⁾ before decapitation.

Mice were decapitated 30 min after the administration of morphine, and the brain and spinal cord were removed within 5 min by complete laminectomy. The brain was dissected according to the method of Glowinski and Iversen¹⁰⁾ into the following divisions: cortex including striatum, dien-+mesencephalon, and pons+medulla oblongata. Spinal cords were dissected into the thoracic and lumbar cord according to the method of Sidman *et al.*¹¹⁾ Three brains or spinal cords were pooled and homogenized, while still frozen, in 3 ml of cold 0.4N perchloric acid. The homogenates were centrifuged at $15000 \times g$ at $2-5^\circ$ for 15 min. From the supernatants, 2.5 ml aliquots were taken and adjusted to pH 7.5-8.5 with 0.4M K_2CO_3 with cooling in an ice bath. Clear supernatant was placed by decantation on doubled columns of aluminum oxide and Amberlite CG-50 for isolating the CA and their metabolites.¹²⁾ The subsequent eluate was determined fluorometrically. P-values were obtained by Student's *t*-test.

Results

As shown in Fig. 1, morphine (10 mg/kg, *s.c.*) had no influence on the 3-MT content in the cortex. 3-MT was not detected in the dien-+mesencephalon and pons+medulla oblongata. 3-MT was detected in the thoracic and lumbar cord in the morphine-treated group but not in the saline-treated group. As indicated in Fig. 2, morphine (10 mg/kg, *s.c.*) did not affect

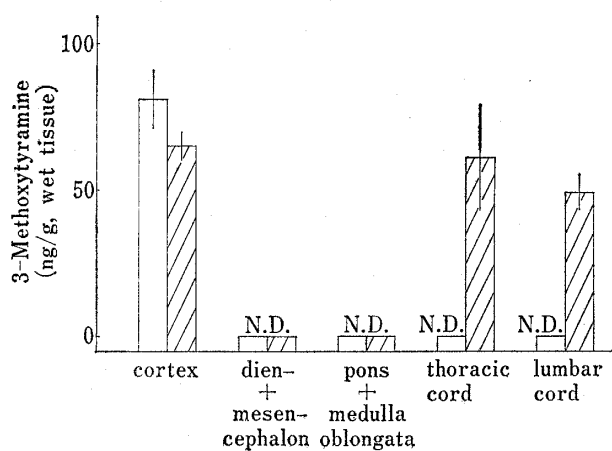


Fig. 1. Effects of Morphine on the 3-Methoxytyramine (3-MT) Content of Various Brain Sites and Two Spinal Parts in Mice

Each vertical column represents the mean of five samples pooled from three brains or spinal cords. Vertical bars represent the S.E. N.D.: no appreciable amount was detected for these samples. □: 0.9% saline. ▨: morphine 10 mg/kg, *s.c.*

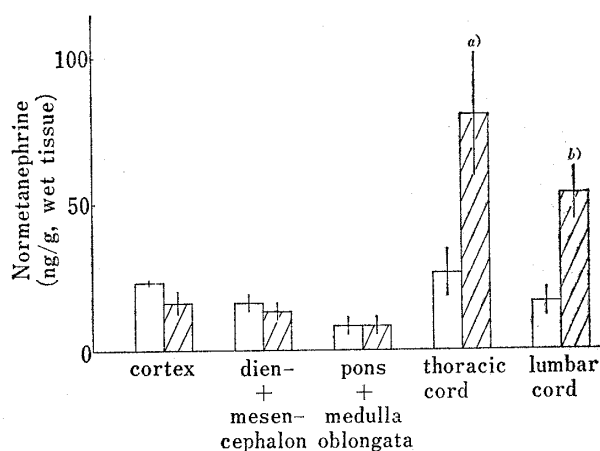


Fig. 2. Effects of Morphine on the Normetanephrine (NM) Content of Various Brain Sites and Two Spinal Parts in Mice

Each vertical column represents the mean of five samples pooled from three brains or spinal cords. Vertical bars represent the S.E. □: 0.9% saline. ▨: morphine 10 mg/kg, *s.c.* Significantly different from the saline-treated control group a) $p < 0.05$; b) $p < 0.01$.

the NM content of the various brain sites compared to the saline-treated group. On the contrary, morphine increased the NM content in the thoracic and lumbar cord compared to the saline-treated group. As shown in Fig. 3, naloxone inhibited the morphine-induced

9) A. Juul, *Arch. Int. Pharmacodyn. Ther.*, **62**, 69 (1939).

10) J. Glowinski and L.L. Iversen, *J. Neurochem.*, **13**, 655 (1966).

11) R.L. Sidman, J.B. Angevine, Jr. and E.T. Pierce, "Atlas of the Mouse Brain and Spinal Cord," Harvard University Press, Cambridge, Massachusetts 1971.

12) T. Karasawa, K. Furukawa, K. Yoshida, and M. Shimizu, *Jpn. J. Pharmacol.*, **25**, 727 (1975).

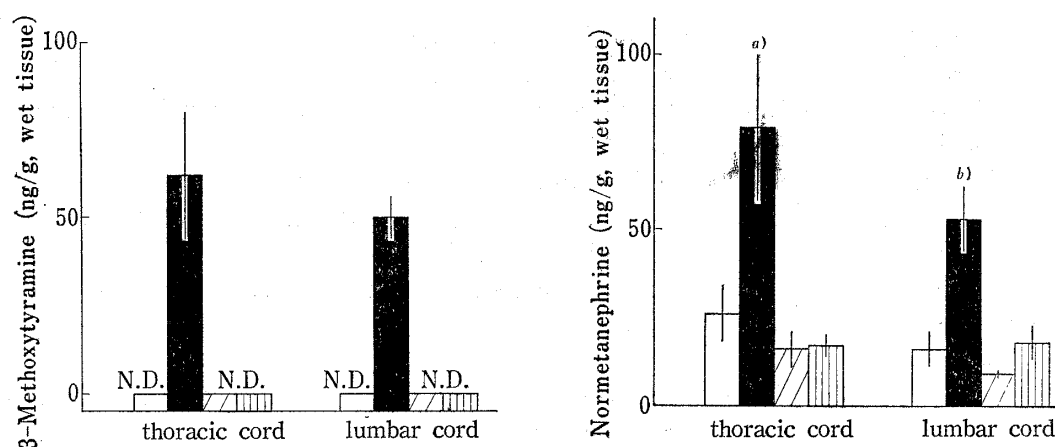


Fig. 3. Effects of Naloxone on the Morphine-Induced Increase in Catecholaminergic Metabolite Content of Thoracic and Lumbar Cord in Mice

Each vertical column represents the mean of five samples pooled from three spinal cords. Vertical bars represent the S.E. □: 0.9% saline. ■: morphine 10 mg/kg, *s.c.* ▨: naloxone 1 mg/kg, *i.p.* ▩: naloxone 1 mg/kg, *i.p.* + morphine 10 mg/kg, *s.c.* Significantly different from the saline-treated control group a) $p < 0.05$; b) $p < 0.01$. N.D.: no appreciable amount was detected for these samples.

increase in 3-MT and NM content in the thoracic and lumbar cord, although naloxone alone had no influence on the NM content compared to the saline-treated group.

Discussion

We have reported that L-dopa (150 mg/kg), L-dopa (150 mg/kg) plus 6-hydroxydopamine (100 μ g/mouse, *i.c.*) and methamphetamine (5 and 10 mg/kg) increase the STR, while phenoxybenzamine (10 mg/kg) and α -methyl-*p*-tyrosine (100 and 400 mg/kg) decrease the STR.⁴ These results indicate that catecholamine in the CNS may facilitate the morphine-induced STR.

It is shown that DA and NE are found in both the brain¹³) and the spinal cord,¹⁴) respectively. In recent years, it has been shown that there is a significant elevation of spinal DA content after spinal injury and α -methyl-*p*-tyrosine prevents trauma-induced DA elevation in the spinal cord.¹⁵) Naftchi *et al.*¹⁶) have shown that an anti-inflammatory agent significantly prevents increased DA content after spinal trauma. Furthermore, it is indicated that DA synthesis in the spinal cord is significantly increased in the traumatized animals.¹⁷) These findings support the possibility that DA as a neurotransmitter may be present in the spinal cord. Adrenergic neurotransmitter in the spinal cord participates tentatively in the regulation of blood pressure, baroreceptor reflex arc,¹⁸) flexor reflex activity¹⁹) and motor activity.²⁰) On the contrary, we have indicated that morphine (10 and 20 mg/kg) by which the STR is elicited undoubtedly does not alter the content of DA and NE in the brain and spinal cord.⁴)

- 13) K. Fuxe, *Acta Physiol. Scand.*, **64**, Suppl., **247**, 39 (1965); O. Lindvall and A. Björklund, *ibid.*, **92**, Suppl., **412**, 1 (1974); D.H.G. Versteeg, J. van der Gugten, J. de Wybren and M. Palkovits, *Brain Res.*, **113**, 563 (1976).
- 14) A. Dahlström and K. Fuxe, *Acta Physiol. Scand.*, **64**, Suppl., **247**, 1 (1965); J.L. Reid, J.A. Zibin, F.H. Foppen and I.J. Kopin, *Life Sci.*, **16**, 975 (1975); J.A. Zibin, J.L. Reid, J.M. Saavedra, and I.J. Kopin, *Brain Res.*, **99**, 293 (1975).
- 15) L.S. Hedeman, M.K. Shellenberger, and J.H. Gordon, *J. Neurosurg.*, **40**, 37 (1974).
- 16) N.E. Naftchi, M. Demey, V. DeCrescito, J.J. Tomasula, E.S. Flamm, and J.B. Campbell, *J. Neurosurg.*, **40**, 52 (1974).
- 17) L. Bunegin, M.S. Albin, and P.J. Jannetta, *Exp. Neurol.*, **53**, 274 (1976).
- 18) J.P. Chalmers and J.L. Reid, *Circ. Res.*, **31**, 789 (1972).
- 19) P. Bolme, H. Corrodi, K. Fuxe, P. Lidbrink, and M. Goldstein, *Eur. J. Pharmacol.*, **28**, 89 (1974).
- 20) N.E. Anden and U. Strömbom, *Psychopharmacologia (Berl.)*, **38**, 91 (1974).

On the other hand, since the O-methylation of CA probably occurs outside the catecholaminergic neurons,⁶⁾ the content of 3-MT and NM probably reflects the amount of DA and NE released by nerve impulses.²¹⁾ In the thoracic and lumbar cord, a sufficient amount of the 3-MT was detected after the administration of morphine (10 mg/kg). The amount of the NM existing in the various brain sites and the spinal cords in mice was well within the range of detection of the method employed. Morphine (10 mg/kg) elevated the NM content in the spinal cord without affecting the NM content in the various brain sites. Shiomi and Takagi²²⁾ have shown that an analgesic dose of morphine increased the content of the NM in the spinal cord of rats. This increasing effect of morphine seems to be common to both mice and rats.

Naloxone which is a specific antagonist of morphine produced a dose-dependent inhibition of the STR due to central excitatory effect of morphine.²³⁾ 3-MT and NM increasing effect of morphine was disappeared by naloxone. These results show that the STR may be elicited by the facilitation of DA and NE release in presynaptic neurons in the spinal cord and that the attenuation of the STR by naloxone is partly due to the inhibition of DA and NE release in the spinal cord in mice. Therefore, the facilitating effects of catecholaminergic agents on the STR might be due primarily to an increased release of CA in the spinal cord in mice, as have already been suggested.⁴⁾

21) N.E. Anden, B. Börjesson, and T. Magnusson, *Europ. J. Pharmacol.*, **4**, 349 (1968).

22) H. Shiomi and H. Takagi, *Brit. J. Pharmacol. Chemother.*, **52**, 519 (1974).

23) T. Kameyama and M. Ukai, *Chem. Pharm. Bull.* (Tokyo), "submitted," (1978).